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L4: Entry 7 of 15

File: PGPB

Apr 17, 2003

PGPUB-DOCUMENT-NUMBER: 20030072794

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030072794 A1

TITLE: Encapsulation of plasmid DNA (lipogenes.TM.) and therapeutic agents with nuclear localization signal/fusogenic peptide conjugates into targeted liposome complexes

PUBLICATION-DATE: April 17, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Boulikas, Teni	Mountain View	CA	US

US-CL-CURRENT: 424/450; 264/4, 435/320.1, 435/458, 514/44

## CLAIMS:

What is claimed is:

1. A method for producing micelles with entrapped therapeutic agents, comprising:  
a) combining an effective amount of a negatively charged therapeutic agent with an effective amount of a cationic lipid in a ratio where about 30% to about 90% the negatively charged atoms are neutralized by positive charges on lipid molecules to form an electrostatic micelle complex in about 20% to about 80% ethanol; and b) combining the micelle complex of step a) with an effective amount of a fusogenic-karyophilic peptide conjugates in a ratio range of about 0.0 to about 0.3, thereby producing micelles with entrapped therapeutic agents.
2. The method of claim 1, wherein the negatively charged therapeutic agent is a therapeutic agent selected from the group consisting of a polynucleotide and a negatively charged drug.
3. The method of claim 2, wherein the polynucleotide is a DNA polynucleotide or an RNA polynucleotide.
4. The method of claim 2, wherein the polynucleotide is a DNA polynucleotide.
5. The method of claim 4, wherein the DNA polynucleotide comprises plasmid DNA.
6. The method of claim 1, further comprising combining an effective amount of an anionic lipid in step a).
7. The method of claim 6, wherein the anionic lipid is dipalmitoyl phosphatidyl glycerol (DDPG) or a derivative thereof.
8. The method of claim 4, further comprising combining an effective amount of a DNA

condensing agent selected from the group consisting of spermine, spermidine, polylysine, polyarginine, polyhistidine, polyornithine and magnesium or a divalent metal ion.

9. The method of claim 5, wherein the plasmid DNA comprises a sequence encoding p53, HSV-tk, p21, Bax, Bad, IL-2, IL-12, GM-CSF, angiostatin, endostatin and oncostatin.

10. The method of claim 1, wherein the cationic lipids are selected from the group consisting of 3.beta.-(N--(N',N'-dimethylaminoethane)carbamoyl)- cholesterol, dimethyldioctadecyl ammonium bromide (DDAB), N-[1-(2,3-dimyristyloxy)propyl]-N,N-dimethyl-N-(2-hydroxyethyl) ammonium bromide (DMRIE), 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP), dioctadecylamidoglycylspermine (DOGS), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), 1,2-dipalmitoyl-3-trimethylammonium propane (DPTAP), 1,2-disteroyl-3-trimethylammonium propane (DSTAP).

11. The method of claim 10, wherein the cationic lipids are combined with the fusogenic lipid DOPE in a molar ratio from about 1:1 to about 2:1.

12. The method of claim 11, wherein the cationic lipids are combined with the fusogenic lipid DOPE in a molar ratio of 1:1.

13. The method of claim 1, wherein the fusogenic-karyophilic peptide is an NLS peptide.

14. The method of claim 13, wherein the NLS peptide is a peptide selected from the group consisting of Seq. ID Nos. 20-622.

15. The method of claim 1, wherein the fusogenic-karyophilic peptide conjugate is a sole fusogenic peptide.

16. The method of claim 1, wherein the NLS peptide component of the fusogenic-karyophilic peptide conjugate is an NLS peptide selected from the group consisting of Seq. ID Nos. 20-622.

17. The method of claim 1, wherein the fusogenic/NLS peptide conjugates comprise amino acid sequences selected from the group consisting of (KAWLKAF).sub.3 (SEQ ID NO:1), GLFKAAAKLLKSLWKLLKA (SEQ ID NO:2), LLLKAFKLLKSLWKLLKA (SEQ ID NO:3) as well as all derivatives of the prototype (Hydrophobic.sub.3Karyophilic.sub.1Hydrophobic.sub.2Karyophilic-1).sub.2-3 where Hydrophobic is any of the A, I, L, V, P, G, W, F and Karyophilic is any of the K, R, or H, containing a positively-charged residue every 3rd or 4th amino acid, that form alpha helices and direct a net positive charge to the same direction of the helix.

18. The method of claim 1, wherein the fusogenic/NLS peptide conjugate comprise an amino acid sequence selected from the group consisting of GLFKAIAGFIKNGWKGMIDGGGYC (SEQ ID NO:4) from influenza virus hemagglutinin HA-2 and YGRKKRRQRRR (SEQ ID NO:5) from TAT of HIV.

19. The method of claim 1, wherein the fusogenic/NLS peptide conjugate comprise an amino acid sequence selected from the group consisting of MSGTFGGILAGLIGLL (K/R/H).sub.1-6 (SEQ ID NO:6), derived from the N-terminal region of the S protein of duck hepatitis B virus but with the addition of one to six positively-charged lysine, arginine or histidine residues, and combinations of these, GAAIGLAWIPYFGPAA (SEQ ID NO:7) derived from the fusogenic peptide of the Ebola virus transmembrane

protein; residues 53-70 (C-terminal helix) of apolipoprotein (apo) All peptide, the 23-residue fusogenic N-terminal peptide of HIV-1 transmembrane glycoprotein gp41, the 29-42-residue fragment from Alzheimer's beta-amyloid peptide, the fusion peptide and N-terminal heptad repeat of Sendai virus, the 56-68 helical segment of lecithin cholesterol acyltransferase.

20. The method of any of claims 13 to 19, wherein the NLS peptide component in fusogenic/NLS peptide conjugates are synthetic peptides containing the above said NLS but further modified by additional K, R, H residues at the central part of the peptide or with P or G at the N- or C-terminus.

21. The method of claim 13, wherein the fusogenic peptide/NLS peptide conjugates are linked to each other with a short amino acid stretch representing an endogenous protease cleavage site.

22. The method of claim 1, wherein the structure of the preferred prototype fusogenic/NLS peptide conjugate used in this invention is: PKKRRGPSP(L/A/I).sub.12-20 (SEQ ID NO:8) where (L/A/I).sub.12-20 is a stretch of 12-20 hydrophobic amino acids containing A, L, I, Y, W, F and other hydrophobic amino acids.

23. The method of claim 1, wherein the fusogenic/NLS peptide conjugates are added to the mixture of DNA/cationic lipid and are incorporated into micelles.

24. The method of claim 1, further comprising combining an effective amount of an encapsulating lipid solution to step b).

25. The method of claim 24, wherein the encapsulating lipid is a lipid comprising cholesterol (40%), dioleoylphosphatidylethanolamine (DOPE) (20%), palmitoyl-oleoylphosphatidylcholine (POPC) (12%), hydrogenated soy phosphatidylcholine (HSPC) (10%), distearoylphosphatidylethanolamine (DSPE) (10%), sphingomyelin (SM) (5%), and derivatized vesicle-forming lipid M-PEG-DSPE (3%).

26. The method of claim 24, wherein the encapsulating lipid is a liposome.

27. The method of claim 26, wherein the liposomes comprises vesicle-forming lipids and between about 1 to about 7 mole percent of distearoylphosphatidyl ethanolamine (DSPE) derivatized with an effective amount of polyethyleneglycol.

28. The method of claim 27, wherein the liposomes have a selected average size of about 80 to about 160 nm.

29. The method of claim 27, wherein the polyethyleneglycol has a molecular weight from about 1,000 to about 5,000 daltons.

30. A micelle with an entrapped therapeutic agent produced by the method of claim 1.

31. A liposome encapsulated therapeutic agent produced by the method of claim 24.

32. The method of claim 31, wherein the therapeutic agent further comprises regulation by a liver, spleen or bone marrow regulatory DNA sequence.

33. The method of claim 32, wherein the regulatory DNA sequence is nuclear matrix DNA isolated from liver, spleen or bone marrow cells.

34. A method for delivering a therapeutic agent in vivo, comprising administration

of an effective amount of the micelle of claim 30 to a subject.

35. The method of claim 34, wherein the therapeutic agent further comprises regulation by a tumor-specific regulatory DNA sequence.

36. The method of claim 35, wherein the tumor-specific regulatory sequence is nuclear matrix DNA isolated from specific tumor cells.

37. A method for delivering a therapeutic agent in vivo, comprising administration of an effective amount of the liposome encapsulated agent of claim 31 to the subject.

38. The method of claim 34 or 37, wherein the administration is intravenous administration or by injection.

39. A micelle with an entrapped DNA polynucleotide produced by the method of claim 9.

40. A method for reducing tumor size in a subject comprising administration of an effective amount of the micelle of claim 39 to the subject.

41. The method of claim 40, further comprising administration of an effective amount of a second therapeutic agent, wherein the agent is selected from the group consisting of ganciclovir, 5-fluorocytosine, an antisense oligonucleotides a ribozyme, and a triplex-forming oligonucleotide directed against genes that control the cell cycle or signaling pathways.

42. The method of claim 41, further comprising administration of an effective amount of a second therapeutic agent, wherein the second therapeutic agent is selected from the group consisting of adriamycin, angiostatin, azathioprine, bleomycin, busulfane, camptothecin, carboplatin, carmustine, chlorambucile, chlormethamine, chloroquinoline sulfonamide, cisplatin, cyclophosphamide, cycloplatam, cytarabine, dacarbazine, dactinomycin, daunorubicin, didox, doxorubicin, endostatin, enloplatin, estramustine, etoposide, extramustinephosphat, flucytosine, fluorodeoxyuridine, fluorouracil, gallium nitrate, hydroxyurea, idoxuridine, interferons, interleukins, leuprolide, lobaplatin, lomustine, mannomustine, mechlorethamine, mechlorethaminoxide, melphalan, mercaptopurine, methotrexate, mithramycin, mitobronitole, mitomycin, mycophenolic acid, nocodazole, oncostatin, oxaliplatin, paclitaxel, pentamustine, platinum-triamine complex, plicamycin, prednisolone, prednisone, procarbazine, protein kinase C inhibitors, puromycine, semustine, signal transduction inhibitors, spiroplatin, streptozotocine, stromelysin inhibitors, taxol, tegafur, telomerase inhibitors, teniposide, thalidomide, thiamiprine, thioguanine, thiotepa, tiamiprine, tretamine, triaziquone, trifosfamide, tyrosine kinase inhibitors, uramustine, vidarabine, vinblastine, vinca alkaloids, vincristine, vindesine, vorozole, zeniplatin, zeniplatin, and zinostatin.

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L4: Entry 14 of 15

File: USPT

Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5854224 A

TITLE: Composition and method for delivery of nucleic acids

Brief Summary Text (81):

To enable the formation of cationic liposomes under appropriate conditions it may be necessary to include the addition of neutral lipids. It is believed that formulation of the invention into liposomes by standard methods with a neutral lipid such as phosphatidylcholine, phosphatidylethanolamine, dioleoylphosphatidylethanolamine (DOPE), DOPC (DOP choline) or cholesterol will increase the capacity of the invention to facilitate delivery of compounds into cells. This is particularly likely where the number of lipophilic groups of the invention is 2. Methods for formulating liposomes are described, for example, in Felgner, P. L. et al. (1987) Proc. Natl. Acad. Sci. (U.S.A.) 84: 7413-7417; Yago, K et al. (1993) Biochem. Biophys. Res. Comm. 196: 1042-1048 and Campbell, M. J. (1995) Biotechniques 18:1027.

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## Refine Search

### Search Results -

Terms	Documents
liposome adj5 (phosphatidylcholine or pc) adj5 (pe or dope) same cationic	15

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<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR</i>			
<u>L4</u>	liposome adj5 (phosphatidylcholine or pc) adj5 (pe or dope) same cationic	15	<u>L4</u>
<u>L3</u>	L2 and 424/450.ccls.	67	<u>L3</u>
<u>L2</u>	L1 and transfect\$	169	<u>L2</u>
<u>L1</u>	liposome same (phosphatidylcholine or pc) same (pe or dope) same cationic	193	<u>L1</u>

END OF SEARCH HISTORY

## Refine Search

### Search Results -

Terms	Documents
liposome same (yeast adj3 transfec\$)	1

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<u>L8</u>	liposome same (yeast adj3 transfec\$)	1	<u>L8</u>
<u>L7</u>	L6 and 424/450.ccls.	2	<u>L7</u>
<u>L6</u>	liposome same yeast same transfec\$	3246	<u>L6</u>
<u>L5</u>	liposome same yeast	4374	<u>L5</u>
<u>L4</u>	liposome adj5 (phosphatidylcholine or pc) adj5 (pe or dope) same cationic	15	<u>L4</u>
<u>L3</u>	L2 and 424/450.ccls.	67	<u>L3</u>
<u>L2</u>	L1 and transfect\$	169	<u>L2</u>
<u>L1</u>	liposome same (phosphatidylcholine or pc) same (pe or dope) same cationic	193	<u>L1</u>

END OF SEARCH HISTORY

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### Search Results -

Terms	Documents
L3 and (cationic adj1 lipid)	24

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<u>L4</u>	L3 and (cationic adj1 lipid)	24	<u>L4</u>
<u>L3</u>	liposome adj5 phosphatidylcholine adj5 (Phosphatidylethanolamine or PE or DOPE)	144	<u>L3</u>
<u>L2</u>	L1 and 424/450.ccls.	30	<u>L2</u>
<u>L1</u>	liposome same transfect\$ same protoplast	5660	<u>L1</u>

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Terms	Documents
L7 and transfect\$	27

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<u>L8</u>	L7 and transfect\$	27	<u>L8</u>
<u>L7</u>	(liposome same \$phosphodiesterase)	86	<u>L7</u>
<u>L6</u>	L3 and 424/450.ccls.	4	<u>L6</u>
<u>L5</u>	L4 and 424/450.ccls.	2	<u>L5</u>
<u>L4</u>	L3 and yeast	2304	<u>L4</u>
<u>L3</u>	(liposome same transfect\$) and \$phosphodiesterase	2445	<u>L3</u>
<u>L2</u>	liposome same phosphodiesterase same transfect\$	4	<u>L2</u>
<u>L1</u>	yeast same phosphodiesterase same transfect\$	8	<u>L1</u>

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### Search Results -

Terms	Documents
L1 and (424/450).ccls.	55

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<u>L2</u>	L1 and 424/450.ccls.	55	<u>L2</u>
<u>L1</u>	liposome same cholesterol same ergosterol	153	<u>L1</u>

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## Refine Search

### Search Results -

Terms	Documents
liposome same \$phosphodiesterase	86

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<i>DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR</i>			
<u>L6</u>	liposome same \$phosphodiesterase	86	<u>L6</u>
<u>L5</u>	liposome adj10 \$phosphodiesterase	5	<u>L5</u>
<u>L4</u>	L3 and yeast	97	<u>L4</u>
<u>L3</u>	liposome same deaminase	204	<u>L3</u>
<u>L2</u>	L1 and 424/450.ccls.	55	<u>L2</u>
<u>L1</u>	liposome same cholesterol same ergosterol	153	<u>L1</u>

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***Your result set for the last L# is incomplete.***

The probable cause is use of unlimited truncation. Revise your search strategy to use limited truncation.

### Search Results -

Terms	Documents
liposome same pc\$ same \$pe same dotap	23

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Search:

liposome same pc\$ same \$pe same dotap

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Hit Count   Set Name  
 result set

DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR

L1   liposome same pc\$ same \$pe same dotap   23   L1

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File: PGPB

Nov 20, 2003

DOCUMENT-IDENTIFIER: US 20030215898 A1

TITLE: Methods for using 22045, a human cyclic nucleotide phosphodiesterase

Detail Description Paragraph:

[0115] A phosphodiesterase polypeptide is also considered to be isolated when it is part of a membrane preparation or is purified and then reconstituted with membrane vesicles or liposomes.

Detail Description Paragraph:

[0303] In general, methods for producing transgenic animals include introducing a nucleic acid sequence according to the present invention, the nucleic acid sequence capable of expressing the protein in a transgenic animal, into a cell in culture or in vivo. When introduced in vivo, the nucleic acid is introduced into an intact organism such that one or more cell types and, accordingly, one or more tissue types, express the nucleic acid encoding the protein. Alternatively, the nucleic acid can be introduced into virtually all cells in an organism by transfecting a cell in culture, such as an embryonic stem cell, as described herein for the production of transgenic animals, and this cell can be used to produce an entire transgenic organism. As described, in a further embodiment, the host cell can be a fertilized oocyte. Such cells are then allowed to develop in a female foster animal to produce the transgenic organism.

Detail Description Paragraph:

[0326] The recombinant host cells are prepared by introducing the vector constructs described herein into the cells by techniques readily available to the person of ordinary skill in the art. These include, but are not limited to, calcium phosphate transfection, DEAE-dextran-mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, lipofection, and other techniques such as those found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

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L2: Entry 28 of 55

File: USPT

Apr 30, 2002

DOCUMENT-IDENTIFIER: US 6379698 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Fusogenic lipids and vesicles

Detailed Description Text (71):

Other lipids that can be incorporated into liposomes of the invention include sterols such as cholesterol or ergosterol, glycolipids such as ganglioside GM.sub.1 (monosialoganglioside) or hydrogenated phosphatidylinositol, acylglycerol lipids such as 1,2-dipalmitoyl-sn-3-succinylglycerol and sphingolipids such as sphingomyelin. In a particular embodiment, liposomes of the invention may comprise from 2-20 mole percent of a glycolipid and 20-95 mole percent of phospholipid, sphingolipid or mixture thereof. In preferred embodiments, where the liposomes also comprise a sterol or acylglycerol, they may comprise 2-20 mole percent (preferably 4-10) of the glycolipid, 40-80 mole percent (preferably 40-80) of phospholipid, sphingolipid or mixture thereof and 10-50 mole percent (preferably 20-40) of sterol or 5-40 mole percent (preferably 10-30) of acylglycerol.

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L2: Entry 35 of 55

File: USPT

Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096720 A

TITLE: Liposomal oligonucleotide compositions

Brief Summary Text (33):

In another preferred embodiment of the invention, the liposomes (B) comprise (i) a glycolipid together with (ii) a vesicle-forming phospholipid or sphingolipid or mixture thereof and, optionally, (iii) a sterol and/or an acylglycerol lipid. The glycolipid is preferably a negatively charged glycolipid, especially ganglioside GM.sub.1 (monosialoganglioside) or hydrogenated phosphatidylinositol. The vesicle-forming phospholipid may be one or more of the phospholipids hereinbefore mentioned, preferably a phosphatidylcholine, a phosphatidylethanolamine or a mixture thereof. Especially preferred phospholipids are distearoyl phosphatidylcholine and dioleoyl phosphatidylethanolamine. The sphingolipid is preferably sphingomyelin and is preferably used together with a phospholipid. The sterol may be, for example, ergosterol or, preferably, cholesterol. The acylglycerol lipid may be an ester of glycerol containing two fatty acid acyl groups each having at least 12 carbon atoms, for example lauroyl, myristoyl, palmitoyl or oleoyl groups, and one acyl group of formula R.sup.1 CO--, where R.sup.1 is a residue, containing up to 10 carbon atoms, of a monocarboxylic acid of formula R.sup.1 COOH after removal of the --COOH group or, preferably, of formula --COR.sup.2 COOH where R.sup.2 is a residue, containing up to 10 carbon atoms, preferably 1 to 4 carbon atoms, of a dicarboxylic acid of formula HOOC--R.sup.2 --COOH, especially succinic acid, after removal of both --COOH groups. An especially preferred acylglycerol is 1,2-dipalmitoyl-sn-3-succinyl glycerol.

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L2: Entry 39 of 55

File: USPT

May 11, 1999

DOCUMENT-IDENTIFIER: US 5902802 A

TITLE: Cationic amphiphiles

Brief Summary Text (38):

Particularly where it is desirable to target a lipid-DNA complex to a particular cell or tissue, a lipid mixture used as a carrier can be modified in a variety of ways. By a lipid mixture is intended a formulation prepared from the cationic amphiphile of the invention, with or without additional agents such as steroids, and includes liposomes, interleaved bilayers of lipid, and the like. Steroids, e.g. cholesterol or ergosterol, can be used in combination with the cationic amphiphiles when used to prepare mixtures. In some embodiments, the lipid mixture will have from 0-67 mole percent steroid, preferably about 33 to 50 mole percent steroid. A lipid-DNA complex is the composition obtained following combination of DNA and a lipid mixture. Non-lipid material (such as biological molecules being delivered to an animal or plant cell or target-specific moieties) can be conjugated through a linking group to one or more hydrophobic groups, e.g. using alkyl chains containing from about 12 to 20 carbon atoms, either prior or subsequent to vesicle formation. Various linking groups can be used for joining the lipid chains to the compound. Functionalities of particular interest include thioethers, disulfides, carboxamides, alkylamines, ethers, and the like, used individually or in combination. The particular manner of linking the compound to a lipid group is not a critical part of this invention, as the literature provides a great variety of such methods. Alternatively, some compounds will have hydrophobic regions or domains, which will allow for their association with the lipid mixture without covalent linking to one or more lipid groups.

Current US Cross Reference Classification (1):

424/450

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File: PGPB

Oct 24, 2002

DOCUMENT-IDENTIFIER: US 20020156259 A1

TITLE: Human adenosine deaminase

Detail Description Paragraph:

[0018] The present invention also includes vectors and expression vectors comprising such nucleic acid molecules. Such expression vectors may comprise a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator. The present invention further includes recombinant host cells, and recombinant viruses, comprising these vectors and expression vectors. Illustrative host cells include avian, bacterial, yeast, fungal, insect, mammalian, and plant cells. Recombinant host cells comprising such expression vectors can be used to produce Zmim19 polypeptides by culturing such recombinant host cells that comprise the expression vector and that produce the Zmim19 protein, and, optionally, isolating the Zmim19 protein from the cultured recombinant host cells. The present invention also includes the protein products of these processes.

Detail Description Paragraph:

[0071] An "adenosine deaminase targeting composition," or a "Zmim19 targeting composition" comprises a Zmim19 moiety (e.g., Zmim19, a Zmim19 fragment, a molecule having Zmim19 activity, and the like) and a recognition molecule. Illustrative recognition molecules include antibodies, antibody components, receptor ligands, and other members of a complement/anti-complement pair. The association between the Zmim19 moiety and the recognition molecule can be covalent or noncovalent. For example, the association between a Zmim19 moiety and a recognition molecule in immunoconjugates and antibody fusion proteins is covalent, while liposomes can comprise a Zmim19 moiety and a recognition molecule in a noncovalent association.

Detail Description Paragraph:

[0113] Different species can exhibit "preferential codon usage." In general, see, Grantham et al., Nuc. Acids Res. 8:1893 (1980), Haas et al. Curr. Biol. 6:315 (1996), Wain-Hobson et al., Gene 13:355 (1981), Grosjean and Fiers, Gene 18:199 (1982), Holm, Nuc. Acids Res. 14:3075 (1986), Ikemura, J. Mol. Biol. 158:573 (1982), Sharp and Matassi, Curr. Opin. Genet. Dev. 4:851 (1994), Kane, Curr. Opin. Biotechnol. 6:494 (1995), and Makrides, Microbiol. Rev. 60:512 (1996). As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (see Table 2). For example, the amino acid threonine (thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequence disclosed in SEQ ID NO:3 serves as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein.

Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

Detail Description Paragraph:

[0156] While the secretory signal sequence of a protein produced by mammalian cells (e.g., tissue-type plasminogen activator signal sequence, as described, for example, in U.S. Pat. No. 5,641,655) is useful for expression of Zmim19 in recombinant mammalian hosts, a yeast signal sequence is preferred for expression in yeast cells. Examples of suitable yeast signal sequences are those derived from yeast mating pheromone .alpha.factor (encoded by the MF.alpha.1 gene), invertase (encoded by the SUC2 gene), or acid phosphatase (encoded by the PHO5 gene). See, for example, Romanos et al., "Expression of Cloned Genes in Yeast," in DNA Cloning 2: A Practical Approach, 2.sup.nd Edition, Glover and Hames (eds.), pages 123-167 (Oxford University Press 1995).

Detail Description Paragraph:

[0186] Zmim19 genes may also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned Zmim19 genes into insect cells. Suitable expression vectors are based upon the Autographa californica multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as Drosophila heat shock protein (hsp) 70 promoter, Autographa californica nuclear polyhedrosis virus immediate-early gene promoter (ie-1) and the delayed early 39K promoter, baculovirus p10 promoter, and the Drosophila metallothionein promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, et al., J. Virol. 67:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, Md.). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Zmim19 polypeptide into a baculovirus genome maintained in E. coli as a large plasmid called a "bacmid." See, Hill-Perkins and Possee, J. Gen. Virol. 71:971 (1990), Bonning, et al., J. Gen. Virol. 75:1551 (1994), and Chazenbalk, and Rapoport, J. Biol. Chem. 270:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Zmim19 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer et al., Proc. Nat'l Acad. Sci. 82:7952 (1985)). Using a technique known in the art, a transfer vector containing a Zmim19 gene is transformed into E. coli, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is then isolated using common techniques.

Detail Description Paragraph:

[0190] Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include Saccharomyces cerevisiae, Pichia pastoris, and Pichia methanolica. Suitable promoters for expression in yeast include promoters from GAL1 (galactose), PGK (phosphoglycerate kinase), ADH (alcohol dehydrogenase), AOX1 (alcohol oxidase), HIS4 (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. These vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YEp vectors such as YEp13 and YCp vectors, such as YCp19. Methods for transforming S. cerevisiae cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Pat. No. 4,599,311, Kawasaki et al., U.S. Pat. No. 4,931,373, Brake, U.S. Pat. No. 4,870,008, Welch et al., U.S. Pat. No. 5,037,743, and Murray et al., U.S. Pat. No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). An illustrative vector system for use in Saccharomyces cerevisiae is the POT1 vector system disclosed by Kawasaki et al. (U.S. Pat. No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators

for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Pat. No. 4,599,311, Kingsman et al., U.S. Pat. No. 4,615,974, and Bitter, U.S. Pat. No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Pat. Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

Detail Description Paragraph:

[0191] Transformation systems for other yeasts, including Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guilliermondii and Candida maltosa are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459 (1986), and Cregg, U.S. Pat. No. 4,882,279. Aspergillus cells may be utilized according to the methods of McKnight et al., U.S. Pat. No. 4,935,349. Methods for transforming Acremonium chrysogenum are disclosed by Sumino et al., U.S. Pat. No. 5,162,228. Methods for transforming Neurospora are disclosed by Lambowitz, U.S. Pat. No. 4,486,533.

Detail Description Paragraph:

[0192] For example, the use of Pichia methanolica as host for the production of recombinant proteins is disclosed by Raymond, U.S. Pat. No. 5,716,808, Raymond, U.S. Pat. No. 5,736,383, Raymond et al., Yeast 14:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming P. methanolica will commonly be prepared as double-stranded, circular plasmids, which can be linearized prior to transformation. For polypeptide production in P. methanolica, the promoter and terminator in the plasmid can be that of a P. methanolica gene, such as a P. methanolica alcohol utilization gene (AUG1 or AUG2). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, the entire expression segment of the plasmid can be flanked at both ends by host DNA sequences. An illustrative selectable marker for use in Pichia methanolica is a P. methanolica ADE2 gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), and which allows ade2 host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, host cells can be used in which both methanol utilization genes (AUG1 and AUG2) are deleted. For production of secreted proteins, host cells can be deficient in vacuolar protease genes (PEP4 and PRB1). Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into P. methanolica cells. P. methanolica cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Detail Description Paragraph:

[0198] Standard methods for introducing expression vectors into bacterial, yeast, insect, and plant cells are provided, for example, by Ausubel (1995).

CLAIMS:

9. A recombinant host cell comprising the expression vector of claim 8, wherein the host cell is selected from the group consisting of bacterium, yeast cell, fungal cell, insect cell, mammalian cell, avian cell, and plant cell.

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L6: Entry 39 of 86

File: PGPB

Jun 27, 2002

DOCUMENT-IDENTIFIER: US 20020081633 A1

TITLE: METHODS FOR USING 22045, A HUMAN CYCLIC NUCLEOTIDE PHOSPHODIESTERASE

Detail Description Paragraph:

[0114] A phosphodiesterase polypeptide is also considered to be isolated when it is part of a membrane preparation or is purified and then reconstituted with membrane vesicles or liposomes.

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L1: Entry 2 of 23

File: PGPB

Jul 28, 2005

DOCUMENT-IDENTIFIER: US 20050163832 A1

TITLE: Intracellular delivery of therapeutic agents

Detail Description Paragraph:

[0049] Liposomes for complexation with DNA did not contain any fluorescent labels, but did contain up to 10 mol % of the cationic lipid DOTAP to enhance plasmid association. Liposomes from a mixture of PC, Ch, DOTAP, and pNP-PEG-PE (7:3:1:0.05 molar ratio) were prepared as above, and incubated with the PEGFP-N1 plasmid overnight at 4.degree. C. In a typical case, the liposome/plasmid complex containing a total of 2 mg lipid and 200 .mu.g DNA was incubated with an appropriate amount of TATp overnight at pH 8.5 in a borate buffer, and purified by gel filtration on Bio-Gel A-1.5. The post-column fraction was subjected to agarose gel electrophoresis to test for the presence and intactness of the plasmid in complex with the liposomes. To determine DNA content, the post-column TATp-liposome/plasmid complex-containing fraction was treated with Triton X-100 for 1 hour at 37.degree. C. to release the plasmid from the complex, and then subjected to agarose gel electrophoresis. Lipofectin.RTM./pEGFP-N1 complex was prepared according to the manufacturer's instruction (Invitrogen Corp.) using same quantities and ratios of lipid and DNA (which are within the recommended limits for this preparation).

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L1: Entry 8 of 23

File: PGPB

Jun 12, 2003

DOCUMENT-IDENTIFIER: US 20030109475 A1

TITLE: Methods and compositions for in vivo gene therapy

Detail Description Paragraph:

[0067] Lipid carriers such as cationic liposomes can mediate high level cellular expression of transgenes or mRNA by delivering the nucleic acid into a wide variety of cells in culture. The use of specific cationic lipids can confer specific advantages for in vivo delivery of complexes. For example, iv injection of nucleic acid complexed to DOTAP-containing liposomes or ethyl-phosphatidylcholine (E-PC) lipid carriers can target transgene expression primarily to the lung. Furthermore, DOTAP, as well as L-PE and cholesterol ester .beta.-alanine (CEBA) are fully metabolized by cells, whereas DOTMA cannot be fully metabolized by cells. Therefore, DOTAP, E-PC, and L-PE, but not DOTMA, are suitable for repeated injection into mammalian hosts. Additionally, using a lipid carrier comprising a cationic lipid and a second lipid, particularly cholesterol or DOPE can maximize transgene expression in vivo. Also, mixing a steroid, such as cholesterol, instead of DOPE, with DOTAP, DOTMA, or DDAB, substantially increases transgene expression in vivo.

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L1: Entry 22 of 23

File: USPT

Oct 27, 1998

DOCUMENT-IDENTIFIER: US 5827703 A

TITLE: Methods and composition for in vivo gene therapy

Detailed Description Text (23):

Cationic liposomes have been shown to be capable of mediating high level cellular expression of transgenes or mRNA by delivering the nucleic acid into a wide variety of cells in culture. The use of specific cationic lipids can confer specific advantages for in vivo delivery. For example, iv injection of nucleic acid complexed to DOTAP-containing liposomes or ethyl-phosphatidylcholine (E-PC) lipid carriers can target transgene expression primarily to the lung. Furthermore, DOTAP, as well as L-PE and CEBA are fully metabolized by cells, whereas DOTMA cannot be fully metabolized by cells. Therefore, DOTAP, E-PC, and L-PE, but not DOTMA, are suitable for repeated injection into mammalian hosts. Additionally, complexing the cationic lipid with a second lipid, primarily either cholesterol or DOPE can maximize transgene expression in vivo. For example, mixing a steroid, such as cholesterol, instead of DOPE with DOTAP, DOTMA, or DDAB, substantially increases transgene expression in vivo.

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